UNIVERSIDADE FEDERAL DO PARÁ
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DAIANE CLAYDES BAÍA DA SILVA

# INFLUENCE OF DIFFERENT IRRIGATION PROTOCOLS ON ROOT CANAL TRANSPORTATION.

BELÉM-PARÁ
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Artigo apresentado ao Programa de Pós-Graduação em Odontologia da Universidade Federal do Pará para obtenção do título de Mestre em Odontologia.

Orientadora: Profa. Dra. Patricia de Almeida Rodrigues da Silva e Souza

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Influência dos diferentes protocolos de irrigação no transporte do canal radicular

Resumo

Introdução: As soluções irrigadoras podem alterar a microdureza dentinária. O objetivo deste estudo foi avaliar, através de microtomografia computadorizada, o transporte do canal radicular pelo uso de quatro diferentes protocolos de irrigação: solução salina, 2.5% NaOCl, mistura de 5% NaOCl+ 18% HEBP e o uso alternado de 2.5%NaOCl e 17% EDTA.

Método: Canais mesiais de 28 molares inferiores humanos com comprimento similar e curvatura entre 25-40° foram divididos randomicamente em 4 grupos (n=14): G1- 0.9% solução salina; G2- 2.5% NaOCl; G3- 2.5% NaOCl/17% EDTA; G4- 5% NaOCl + 18% HEBP. O escaneamento das raízes por sistema µCT com uma resolução isotrópica de 19µm foi realizado pré e pós instrumentação do canal com a lima Primary Wave One. As imagens foram usadas para medir o transporte do canal nos terços cervical, médio e apical. Os dados foram submetidos ao teste estatístico não paramétrico Kruskal–Wallis ($\alpha<0.05$), a fim de comparar os grupos e para comparação dos terços radiculares em cada grupo.

Resultados: O transporte do canal ocorreu em todos os grupos experimentais. Não sendo observada diferença estatística entre os grupos (p.0.05). Entretanto, os terços cervical e médio mostraram maior transporte quanto comparados ao terço apical ($p>0.05$). Nos terços cervical e médio o transporte ocorreu para a região de furca, enquanto no terço apical o transporte ocorreu para mesial.

Conclusão: O uso do hipoclorito de sódio e de agentes quelantes em diferentes protocolos de irrigação durante a instrumentação não aumentou significantemente o transporte do canal radicular.

Palavras-chaves: EDTA, ácido etidronico, hipoclorito de sódio, transporte, imagens de microtomografia computadorizada

**SUMÁRIO**

Influence of different irrigation protocols on root canal transportation.

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# Influence of different irrigation protocols on root canal transportation.


## Abstract

Introduction: The irrigants solutions can alter dentin microhardness. The aim of this study was to evaluate, throught computed microtomography, the root canal transportation caused by the use of four different irrigation protocols: saline, 2.5%NaOCl, 5%NaOCl+18%HEBP mixture and alternating use of 2.5%NaOCl and 17%EDTA.

Methods: Mesial canals of 28 human mandibular molar with similar length and curvature ranging from 25-40° were randomly distributed into 4 groups (n=14): G1- 0.9% saline solution; G2- 2.5% NaOCl; G3- 2.5% NaOCl/17% EDTA; G4- 5% NaOCl + 18% HEBP. Scanning of roots by µCT system with an isotropic resolution of 19µm were carried out pre- and postpreparation of the canals with Primary Wave One file. The images were used to measure the root canal transportation at the cervical, middle and apical third. The data were subjected to the nonparametric statistical Kruskal–Wallis test ($p<0.05$) to compare the different groups and to comparison of the root thirds in each group.

Results: The root canal transportation occurred in all experimental groups. No significant differences was observed between groups ($p>0.05$). However, the cervical and middle third showed the higher transportation in comparison with apical third($p<0.05$). In the cervical and middle third the transportation occurred to furcation, while in the apical third the transportation occurred to mesial.

Conclusions: The use of hypochlorite sodium and chelating agents in different irrigating protocols during instrumentation did not increase significantly the root canal transportation.


Keywords: EDTA, etidronic acid, sodium hypochlorite, transportation, x-ray micro-computed tomographic imaging

Introduction

The sodium hypochlorite solution (NaOCl) is the most widely used irrigant during root canal treatment (1, 2-4), due its antimicrobial activity and unique tissue-dissolving capacity (1, 2, 3). However, NaOCl is unable to dissolve inorganic components of smear layer (1, 2-4) formed during the instrumentation of the canal (5); therefore, the use of chelating agents is necessary (2, 3) such as sodium salt (2), EDTA, citric acid (1-3, 5) and 1-hydroxyethtylidene-1,1-bisphosphanoate (HEBP) (1, 3, 6).

HEBP is a weak-chelating agent with less damage to dentine (7, 8) and can be mixed with NaOCl (3, 7), without interfering with its capacity of tissue-dissolving (3, 9) and antimicrobial properties (9-11).

Irrigating agents interfere in the chemical structure of human dentine (5, 6, 12), modifying the calcium/phosphorus (Ca/P) ratio of the dentin surface (6, 12), which alters its physical and mechanical properties (5, 6), and may, thereby, increase its permeability, solubility, surface roughness, and decrease its microhardness (5, 6, 12). The chelating agents cause the greatest reduction in dentin microhardness which can influence in the maintenance of the original path of the canal, causing the transportation of the root canal (6, 13, 14).

To investigate the transportation in curved root canals, several methods have been used, including the use of digital radiographs, computed tomography and the analysis of root cross sections (6, 15). The high-resolution microcomputed tomography scanning (μCT) has been widely used in studies of transport of the root canal (14-17) and has the advantage of being a nondestructive method that allows the comparison of the anatomical canal before and after instrumentation (15, 18).

Considering the dentinal alterations promoted by irrigating solutions and the possibility of them to affect the maintenance of the root canal path during the instrumentation, the primary aim of this study was to evaluate the root canal transportation by using four different irrigation protocols: saline (control), 2.5%NaOCl, 5%NaOCl+18%HEBP mixture and the use of 2.5%NaOCl and 17%EDTA alternately. The secondary objective was to compare whether there were differences among the root thirds and evaluate the direction of deviation. The null hypothesis was that the root canal transportation increases when chelating agents are used during the instrumentation of curved canals.

## Materials and Methods

### Solutions

Saline solution, 17% EDTA, 18% HEBP, 2.5% and 5% NaOCl were used in the present study. HEBP salt (Zschimmer & Schwarz, Mohsdorf, Germany) and pure chemicals of NaOCl (Sigma-Aldrich. St. Louis, MO, USA) was diluted in distilled water to 18% HEBP, 2.5% and 5% NaOCl respectively. To obtain the 17% EDTA solution, the disodium EDTA (Sigma-Aldrich) was dissolved in distilled water with aid of sodium hydroxide (NAOH) (Sigma-Aldrich).

The solutions were stored at 5°C in airtight dark containers. Immediately before the experiment the solutions were taken out from the refrigerator and stored for 60 min at room temperature. Then, a fresh 1:1 mixture of 5%NaOCl and 18% HEBP was prepared, producing a solution that contained 2.5%NaOCl and 9% HEBP.

## Sample selection

After Research Ethics Committee approval (CEP 1.044.287) twenty-eight human mandibular molars were selected according the following criteria: teeth with mature apices, mesial roots with two canals, distinct apical foramens, similar length, curvatures ranging from 25° and 40° (19) and the apical diameter compatible with a size #10 k-file. Teeth root surfaces were cleaned with hand curettes and stored in saline solution at 5° C. This selection resulted in 56 samples.

## µCT Scanning Procedures

The teeth were scanned using a high- definition µCT system (SkyScan 1174v2; Bruker-microCT, Kontich, Belgium) at 50 kV and 800 µA, 19 µm voxel size, 360° rotation around the vertical axis and rotation step of 1.0°. Their crowns were inserted into a custom-made silicone mold (Zetaplus; Labordental, São Paulo, Brazil) before scanning, to allow exact reposition of mesial root for post-instrumentation scanning at the same initial position.

Image reconstructions were made using and specific software (NRecon v.1.6.3, Bruker-microCT) and then, they were saved in axial sections in BMP format for 3D models obtainning (CTAn v.1.14.4; Bruker-microCT).

## Root canal preparation

Conventional coronal access procedures were performed and the distal roots were removed using a low-speed diamond disk (kG Soarensen, Barueri, Brazil) with cooling system. Mesiobuccal and mesiolingual canals were located and a size #10 k-file (Dentsply

Maillefer, Ballaigues, Switzerland) was used for the glide path. The working length was established inserting a #10 K-file until its tip could be seen through the apical foramen, and the working length was determined subtracting 1.0 mm from this length.

Root canals (n=56) were randomly assigned to four groups according to the irrigation protocol.

- Group 1 (G1): Irrigation with 6 mL of 0.9% saline solution (control).
- Group 2 (G2): Irrigation with 6 mL of 2.5% NaOCl
- Group 3 (G3): Irrigation with 6 mL of 5% NaOCl solution and 18% HEBP mixed in equal parts.
- Group 4 (G4): Alternating irrigation with 2.5% NaOCl and 17% EDTA. During instrumentation, the canal was filled with NaOCl, and between each instrument change, the canal was filled with 1mL of EDTA for 1 min.

In all groups, the canals were instrumented using a Wave One Primary file (Dentsply Maillefer) operated in a X-Smart Plus electric motor (Dentsply Maillefer). according to the manufacture’s recommendations. After 3 pecking motions, the instrument was removed and cleaned, and the canal was irrigated with 2 ml of the tested solutions. The total instrumentation and irrigant contact time for each canal was standardized in 10 min, only for group 4 this time was increased in 3 min for EDTA application.

The irrigation was carried out using a disposable plastic syringe attached to a 30-gauge NaviTip needle (Ultradent, South Jordan, UT) placed as close as possible to the working length (1–2 mm). After all shaping procedures, a new µCT scan was performed using the same parameters.

Evaluation of root canal transportation

Different perpendicular cross-sectional planes of image before and after instrumentation at 1, 2 and 3mm from the apical foramen; below the furcation level and in middle level were evaluated to compare the canal transportation by Gambill's et al. (20) technique. The measuring the shortest distance from the edge of uninstrumented canal to the periphery of the root (mesial=a1; distal=b1) was compared with the same measurements obtained from the instrumented images (mesial=a2; distal=b2) (Figure 1), then the amount of canal transportation was determined using the following formula: $|(a1 - a2) – (b1– b2)|$. For data analysis, the mean value of the measurements obtained in each third were used.

Values of 0 indicated that there was no canal transportation; positive values indicated transportation for mesial; and negative value indicated transportation to distal.

Statistical Analysis

For sample distribution, the One-way of variance was used to assess the homogeneity of the groups regarding the curvature angle, length and volume before root canal treatment.

The Shapiro-Wilk test was used to verify the normality of root canal transportation data. The data did not follow a normal distribution; thus, the nonparametric Kruskal–Wallis and Dunn's tests ($\alpha<0.05$) were used to compare the different groups and comparison of the root canal thirds in each group.

## Results

The median and interquartile range of transportation are shown in Table 1. The root canal transportation occurred in all experimental groups; however, statistical analysis did not detected significant difference among the groups G1, G2, G3 and G4. However, the cervical and middle third showed higher transportation compared with the apical third ($p>0.05$) (Table 2).

A descriptive analysis showed a greater percentage of transportation for distal (furcation) direction in cervical and middle level and for mesial direction in the apical level (Table 3).

## Discussion

Root canal transportation results in untouched areas that can perpetuate infection by harboring organic matter, debris and contaminated dentine (21), leading to failure of the endodontic treatment. This accident occurs during the root canal treatment and is influenced by instrumentation technique, type of file, chelating agents and irrigating solution (6, 13).

This study evaluated the potential influence of irrigation protocols in transport of curved root canals during instrumentation. The hypothesis was based on previous studies, that found a change in dentin microhardness, through the use of irrigating solutions, especially after the application of chelating agents (22-24). The null hypothesis tested was rejected once the use of chelating agents did not increase the root canal transportation.

Efforts were carried out to pair samples, in order to obtain groups with similar anatomy. Therefore, similar mesial mandibular roots with two canals and distinct foramens were distributed in groups considering apical diameter (compatible K 15 file), volume of the apical portion (1.26 to 8.11), canal length (19 to 21 mm) and the angle of canal curvature (25° to 40°).

The most widely used protocol for preparing root canal has been the use of NaOCl and EDTA. However, studies have shown harmful effects dentin (6, 23, 25) in order to reduce such effects has been tested using sodium hypochlorite associated with HEBP (1, 3, 6, 7-12), a weak-chelating agent with less damage to dentine (7, 8) and has the advantage of being able to be mixed with the NaOCl (3, 7) without interfering with its capacity to tissue-dissolving (3, 9) and antimicrobial properties (9-11) reducing the formation of smear layer and the clinical working time.

It has been shown that the use of EDTA as irrigating agent during the instrumentation with NiTi hand files increased the apical transportation, when compared to canals irrigated with distilled water, probably because of the removal capacity of calcium ions of dentin by EDTA, altering dentinal physical and mechanical properties, making the dentine smoother and more easily removable (13). Even when HEBP, weak-chelating agent with less damage to dentine (7, 8), was used as irrigant, in conjunct with Protaper Universal, the apical transportation was observed (6).

The experimental groups in this research, regardless the irrigation solution used, demonstrated the same behavior with regard to deviation from the original canal path. The median transportation values range from 0.05 to 0.06 in apical third, 0.12 to 0.18 and 0.21 to 0.23 in middle and cervical level respectively. The clinical relevance of maximum transportation value remains questionable. Values up to 0.10 mm are considered acceptable (26), but was reported the negative impact of apical transportation of more than 0.3mm on the root filling, with values of transportation > 0.3 mm showing more frequently leakage (27). In apical third, all samples showed values below this limit. Ten samples of the cervical third (3 G1, 3 G2, 2 G3 and 2 G4) showed transportation above 0.3 mm in relation to the middle third of the transportation beyond this limit were observed in two samples (2 G4).

When comparing studies where the influence of irrigation solutions on the deviation of the root canal was evaluated, differences are found in the type of analysis, the type of instrument and the solutions time of action (6, 13, 14). The manual instrumentation and rotation systems require more instrumentation time in comparison to single file reciprocating systems. Thus, there is a longer contact time between the irrigating solution and dentin, and the prolonged action of chelators and sodium hypochlorite may result in higher decalcification of the dentine (28) and increased area for chelator agents action due to the deproteination of the canal by NaOCl, which facilitates the cutting of the dentin by decrease of microhardness dentine (6).

In this study, the canal root transportation occurred in all groups without influence of the type of irrigation. Therefore, this is a result of the action of Wave One Primary file. The cervical ($p<0.05$) and middle third ($p<0.05$) showed the higher transportation in comparison with apical third. The direction of transportation in the cervical and middle third occurred towards the furcation and in the apical third to mesial. These results corroborate with a previous study that evaluated, by microcomputed tomography, the transport of instrumented root canal by Primary Wave One with and without prior cervical and apical enlargement, showing that the direction of transportation in cervical and middle level was to furcation, whereas the apical thirds were transported to mesial, without significant difference between cervical and middle level of the root (16).

The greatest amount of dentine removed toward furcation in cervical third also was reported using the Protaper Universal, k3 (29), stainless steel (30), Mtwo and Reciproc (31). The channel anatomy can explain this effect, by the presence of cervical dentin projection, that forces the instrument in reverse direction causing increased tension and wear in the region of furcation in the middle-coronal level (29).

Differences among the root thirds also may be due to the characteristics of the system used. Wave One system, made of nickel-titanium alloy M-Wire, has different progressive taper increasing, inactive tip and varied cross-section along the active part (17, 32), convex triangular in the middle and coronary file portion and convex triangular modified with radial land in the apical portion (16), which gives the instrument more centralized capacity (17, 32). The largest diameter of this file in the middle and cervical portions provide less flexibility in relation to the apical portion, which may be responsible for the largest deviation in the cervical and middle thirds when compared to the apical third.

Therefore, this study demonstrated that the use of sodium hypochlorite and chelating agents used in different irrigating protocols during instrumentation with Wave One system did not significantly increase the root canal transportation. The transportation related to the action of Primary Wave One file was higher at cervical and middle thirds compared to the apical third; this transportation was directed to the danger zone in the cervical and middle thirds and to mesial in the apical third.

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Figure Legends

Figure 1. Tooth micro-CT sections of apical regions of unistrumented (A) and instrumented (B) canals to evaluate apical root canal transportation.

TABLE 1. Median, Minimal (Min) and Maximum (Max) values of the root canal transportation comparisons in millimeters.

| Groups | Cervical | Middle | Apical |
|---|---|---|---|
| | Median (Min – Max) | Median (Min – Max) | Median (Min – Max) |
| G1 | 0.21 (0.13 - 0.43) | 0.16 (0.09 - 0.24) | 0.05 (0.01 - 0.12) |
| G2 | 0.22 (0.09 - 0.45) | 0.12 (0.04 - 0.22) | 0.05 (0.03 - 0.08) |
| G3 | 0.23 (0.16 – 0.35) | 0.18 (0.07- 0.27) | 0.05 (0.01 - 0.10) |
| G4 | 0.23 (0.05 – 0.33) | 0.15 (0.09- 0.30) | 0.06 (0.01 - 0.12) |
| p-value | 0.8152 | 0.2308 | 0.5899 |

G1 – saline; G2 – NaOCl; G3 – NaOCl associated with HEBP; G4 –NaOCl alternate with EDTA.

TABLE 2. Rank difference and p-value (Kruskal-Wallis) to compare root canal transportation between root canal levels

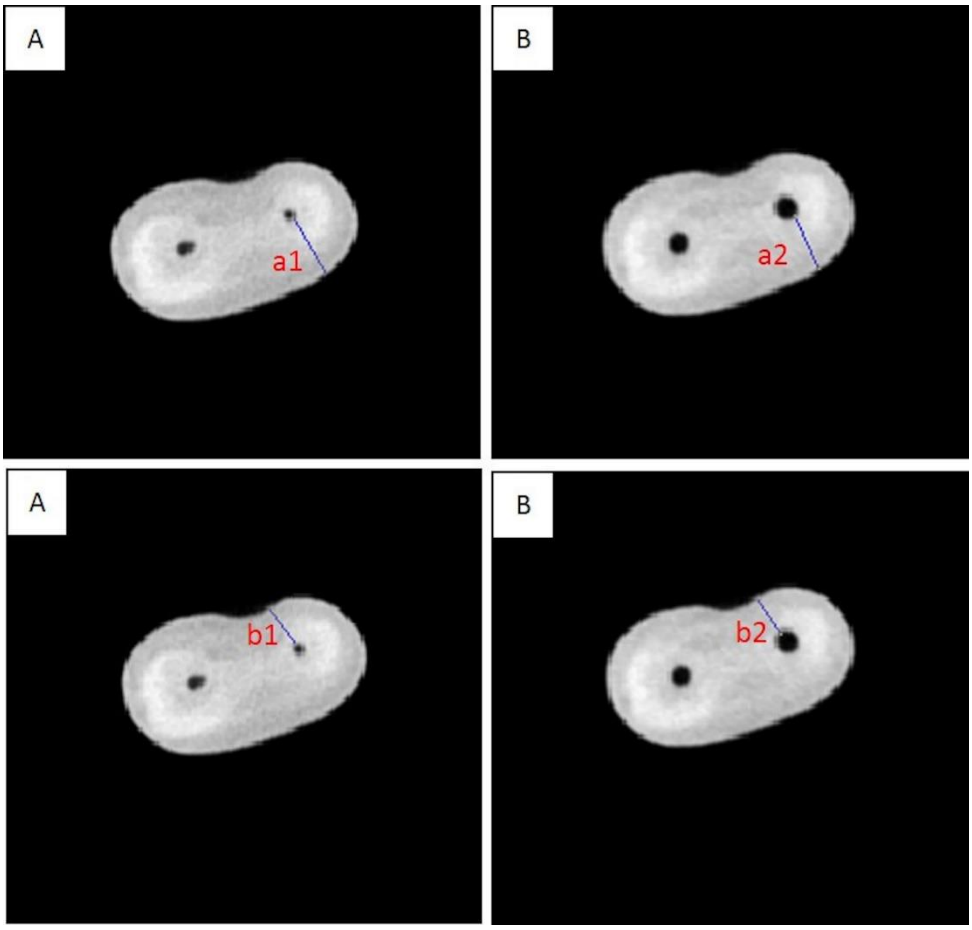
| Level | G1 | | G2 | | G3 | | G4 | |
|---|---|---|---|---|---|---|---|---|
| | Rank diference | p-value | Rank diference | p-value | Rank diference | p-value | Rank diference | p-value |
| Cervical x Middle | 9.18 | ns | 11.60 | Ns | 9.43 | ns | 6.36 | ns |
| Cervical x Apical | 24.25 | <0.05 | 25.04 | <0.05 | 25.29 | <0.05 | 22.04 | <0.05 |
| Middle x Apical | 15.07 | <0.05 | 13.43 | <0.05 | 15.86 | <0.05 | 15.68 | <0.05 |

G1 – saline; G2 – NaOCl; G3 – NaOCl associated with HEBP; G4 –NaOCl alternate with EDTA; $p < 0.05$.

TABLE 3. Direction of root canal transportation in each root canal level.

| Groups | Direction | Cervical | | Middle | | Apical | |
|---|---|---|---|---|---|---|---|
| | | n | % | n | % | n | % |
| G1 | Mesial | 19 | 45,20 | 13 | 30,95 | 25 | 59,52 |
| | Distal | 22 | 52,38 | 29 | 69,05 | 14 | 33,33 |
| | Nulo | 1 | 2,38 | 0 | 0,00 | 3 | 7,14 |
| G2 | Mesial | 15 | 35,71 | 11 | 26,19 | 25 | 59,52 |
| | Distal | 27 | 64,29 | 30 | 71,43 | 16 | 38,10 |
| | Nulo | 0 | 0,00 | 1 | 2,38 | 1 | 2,38 |
| G3 | Mesial | 18 | 42,86 | 10 | 23,81 | 23 | 54,76 |
| | Distal | 23 | 54,76 | 32 | 76,19 | 19 | 45,24 |
| | Nulo | 1 | 2,38 | 0 | 0,00 | 0 | 0,00 |
| G4 | Mesial | 20 | 47,62 | 17 | 40,48 | 22 | 52,38 |
| | Distal | 21 | 50,00 | 25 | 59,52 | 18 | 42,86 |
| | Nulo | 1 | 2,38 | 0 | 0,00 | 2 | 4,76 |

Figure 1. Tooth micro-CT sections of apical regions of unistrumented (A) and instrumented (B) canals to evaluate apical root canal transportation.



Influência de diferentes protocolos de irrigação no transporte canal radicular.

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# Influência dos diferentes protocolos de irrigação no transporte do canal radicular

Resumo

Introdução: As soluções irrigadoras podem alterar a microdureza dentinária. O objetivo deste estudo foi avaliar, através de microtomografia computadorizada, o transporte do canal radicular pelo uso de quatro diferentes protocolos de irrigação: solução salina, 2.5% NaOCl, mistura de 5% NaOCl+ 18% HEBP e o uso alternado de 2.5%NaOCl e 17% EDTA. Método: Canais mesiais de 28 molares inferiores humanos com comprimento similar e curvatura entre 25-40° foram divididos randomicamente em 4 grupos (n=14): G1- 0.9% solução salina; G2- 2.5% NaOCl; G3- 2.5% NaOCl/17% EDTA; G4- 5% NaOCl + 18% HEBP. O escaneamento das raízes por sistema µCT com uma resolução isotrópica de 19µm foi realizado pré e pós instrumentação do canal com a lima Primary Wave One. As imagens foram usadas para medir o transporte do canal nos terços cervical, médio e apical. Os dados foram submetidos ao teste estatístico não paramétrico Kruskal–Wallis ($\alpha<0.05$), a fim de comparar os grupos e para comparação dos terços radiculares em cada grupo. Resultados: O transporte do canal ocorreu em todos os grupos experimentais. Não sendo observada diferença estatística entre os grupos (p.0.05). Entretanto, os terços cervical e médio mostraram maior transporte quanto comparados ao terço apical ($p>0.05$). Nos terços cervical e médio o transporte ocorreu para a região de furca, enquanto no terço apical o transporte ocorreu para mesial. Conclusão: O uso do hipoclorito de sódio e de agentes quelantes em diferentes protocolos de irrigação durante a instrumentação não aumentou significantemente o transporte do canal radicular.

Palavras-chaves: EDTA, ácido etidronico, hipoclorito de sódio, transporte, imagens de microtomografia computadorizada

Introdução

A solução de hipoclorito de sódio (NaOCl) é o irrigante comumente usado durante o tratamento do canal radicular (1, 2-4), devido a sua atividade antimicrobiana e sua capacidade única de dissolução tecidual (1, 2, 3). No entanto, NaOCl é incapaz de dissolver os componentes inorgânicos da camada de smear layer (1, 2-4), formado durante a instrumentação do canal (5); sendo necessário o uso de agentes quelantes (2, 3), tais como sal de sódio (2), EDTA, ácido cítrico (1-3, 5) e 1-hidroxietilideno-1,1-bifosfonato (HEBP) (1, 3, 6).

HEBP é um agente quelante fraco com menor dano a dentina (7, 8) e pode ser misturado com o NaOCl (3, 7), sem interferir com a sua capacidade de dissolução tecidual (3, 9) e propriedades antimicrobianas (9-11).

Os agentes irrigantes interferem na estrutura química da dentina humana (5, 6, 12) alterando a relação cálcio / fósforo (Ca / P) da superfície dentinária (6, 12), o que altera suas propriedades física e mecânicas (5, 6), podendo aumentar a permeabilidade, solubilidade e a rugosidade superficial e reduzir a microdureza dentinária (5, 6, 12). Os agentes quelante causam maior redução na microdureza dentinária e essa mudança na propriedade da microdureza pode influenciar na manutenção da trajetória original do canal causando o transporte do canal radicular (6, 13, 14).

Para investigar o transporte em canais radiculares curvos inúmeros métodos tem sido usado, incluindo o uso de radiografias digitais, tomografia computadorizadas e análise de seções transversais de raiz (6, 15). O escaneamento de microtomografia computadorizada (µCT) de alta resolução, tem sido amplamente utilizado em estudos de transporte de canal radicular (14-17) e tem a vantagem de ser um método não destrutivo que permite a comparação da anatomia do canal antes e após a instrumentação (15, 18).

Considerando as alterações dentinárias promovidas pelas soluções irrigadoras e a possibilidade que essas afetem a manutenção da trajetória do canal radicular durante a instrumentação, o objetivo principal desse estudo foi avaliar o transporte do canal radicular pelo uso de quatro diferentes protocolos de irrigação: solução salina (controle), 2.5% NaOCl, mistura de 5% NaOCl+ 18% HEBP e o uso alternado de 2.5%NaOCl e 17% EDTA. O objetivo secundário foi comparar se houve diferenças entre os terços radiculares e avaliar a direção do desvio. A hipótese experimental foi o aumento do transporte do canal radicular quando agentes quelantes são usados durante a instrumentação de canais curvos.

## Materiais e métodos

### Soluções

Solução salina, 17% EDTA, 18% HEBP, 2.5% e 5% NaOCl foram usados no presente estudo. Sal de HEBP (Zschimmer & Schwarz Mohsdorf GmbH & Co KG, Burgstädt, Alemanha) e produtos químicos puros de NaOCl (Sigma-Aldrich. St. Louis, MO, EUA) foram diluídos em água destilada a 18% HEBP, 2,5% e 5% NaOCl respectivamente. Para obter soluções de 17% de EDTA (Sigma-Aldrich) a dissódico de EDTA (Sigma-Aldrich) foi dissolvendo em água destilada com o auxílio da adição de hidróxido de sódio (NaOH) (Sigma-Aldrich); ácido clorídrico (HCl) (Sigma-Aldrich) foi adicionado para ajustar o pH para 7.0.

As soluções foram armazenadas a 5°C em recipientes escuros hermeticamente fechados. Imediatamente antes da experiência, as soluções foram retiradas do refrigerador e armazenado durante 60 min a temperatura ambiente e uma mistura fresca de 1: 1 de 5%

de NaOCl a 18% HEBP foi preparado, produzindo uma solução que continha 2,5% de NaOCl e 9% de HEBP.

Seleção da amostra

Após a aprovação pelo Comitê de Ética em Pesquisa (CEP 1.044.287) vinte e oito molares inferiores humano foram selecionados de acordo com os seguintes critérios: dentes com ápices fechado, raízes mesiais com dois canais, forames apicais distintos, comprimento semelhante, curvaturas variando entre 25 ° e 40 ° (19) e de diâmetro apical compatível com uma lima k 10. As superfícies radiculares dos dentes foram limpas com curetas manuais e armazenadas em solução salina a 5 ° C. Dessa seleção resultaram 56 amostras.

Processo de escaneamento µCT

Os dentes foram escaneados usando um sistema µCT de alta definição (SkyScan 1174 v 2; Bruker-micro CT, Kontich, Bélgica) a 50 kV e 800 µA, 19 µm tamanho de voxel, rotação de 360 ° em torno do eixo vertical e passo de rotação de 1.0 °. As coroas foram inseridas em um molde de silicone pré-fabricado (Zetaplus; Labordental, São Paulo, Brasil) antes do escaneamento, para permitir o reposicionamento da raiz mesial para o escaneamento pós-instrumentação na mesma posição inicial.

A reconstrução das imagens foram feitas usando software específico (NRecon v.1.6.3, Bruker-microCT) e em seguida salvas em cortes axiais em formato BMP para obtenção dos modelos 3D (CTAN v.1.14.4; Bruker-microCT).

Preparo do canal radicular

Acesso coronário convencional foi realizado e as raízes distais foram removidas usando disco de diamante sob baixa rotação (KG Sorensen Ind. E Com., Barueri, Brasil), com sistema de refrigeração. Canais mésio-vestibulares e mésio-lingual foram localizados e explorados com uma lima k 10 (Dentsply Maillefer, Ballaigues, Switzerland) para garantir a patência. O comprimento de trabalho (CT) foi estabelecido inserindo uma lima K 10 até sua ponta poder ser vista através do forame apical, e o comprimento de trabalho determinado subtraindo 1,0 mm deste comprimento.

Canal radiculares (n=56) foram randomicamente divididos em quatro grupos de acordo com o protocolo de irrigação.

- Grupo 1 (G1): Irrigação com 6mL de 0.9% de solução salina (controle)
- Grupo 2 (G2): Irrigação com 6mL de 2.5% NaOCl
- Grupo 3 (G3): Irrigação com 6mL de solução de 5% NaOCl e 18%HEBP, misturados em proporções iguais.
- Grupo 4 (G4): Irrigação alternada com 2.5% NaOCl e 17% EDTA. Durante a instrumentação, o canal foi preenchido com NaOCl e entre cada troca de instrumento o canal foi preenchido com 1mm de EDTA por 1mm.

Todos os canais foram instrumentados com a lima Wave One Primary (Dentsply Maillefer) operada em um motor elétrico X-Smart Plus (Dentsply Maillefer) de acordo com o manual do fabricante. Depois de 3 movimentos de bicada, o instrumento foi removido e limpo, e o canal foi irrigada com 2 ml de solução. A instrumentação total e tempo de contato do irrigante para cada canal foi padronizado para 10 min, apenas para o grupo 4 foi aumentado 3 min para aplicação EDTA.

A irrigação foi realizada utilizando uma seringa de plástico descartável acoplada a uma agulha NaviTip (Ultradent, South Jordan, UT) de 30-gauge, posicionada tão próximo possível ao comprimento de trabalho (1-2 mm). Após o procedimento de modelagem, um novo escaneamento com a µCT foi realizado usando os mesmos parâmetros.

Avaliação do transporte do canal radicular

Diferentes planos de secção transversal das imagens antes e após a instrumentação a 1, 2 e 3 milímetros do forame apical; do nível de bifurcação e no terço médio foram avaliados para comparar o transporte do canal pela técnica de Gambill's et al. (20). A menor distância da borda mesial da curvatura da raiz a borda mesial do canal não instrumentado (mesial=a1; distais=b1) foi comparado com as mesmas medidas obtidas a partir das imagens dos canais instrumentados (mesial = A2; distai = b2) (Figura 1) , então a quantidade de transporte do canal foi determinada utilizando a seguinte fórmula: | (A1 - A2) - (B1- B2)| Para a análise dos dados utilizou-se a média das medidas obtidas em cada terço.

Valores igual a 0 indica que não ocorreu transporte do canal, valores positivos indica desvio para mesial e valores negativos para distal.

Análise estatística

Para a distribuição da amostra utilizou-se o ANOVA para avaliar a homogeneidade dos quatro grupos em relação ao ângulo de curvatura, comprimento e volume antes do tratamento do canal.

O teste de Shapiro-Wilk foi utilizado para verificar a normalidade dos dados do transporte do canal radicular. Os dados não mostraram distribuição normal; assim teste não paramétrico de Kruskal-Wallis ($\alpha < 0,05$) foi utilizado para comparar os diferentes entre os grupos e na comparação dos terços do canal radicular.

## Resultados

A mediana e o desvio interquartil do transporte são apresentados na Tabela 1. O transporte do canal radicular ocorreu em todos os grupos experimentais; entretanto, a análise estatística não detectou diferença significativa entre os grupos G1, G2, G3 e G4. No entanto, os terços cervical e médio mostraram transporte mais elevados em comparação com o terço apical ($p > 0,05$) (Tabela 2).

A análise descritiva mostrou uma maior percentagem de transporte para a distal (região de furca) nos níveis cervical e médio e para mesial no terço apical (Tabela 3).

## Discussão

O transporte do canal radicular resulta em áreas intocadas que podem perpetuar a infecção por abrigar matéria orgânica, detritos e contaminação da dentina (21), levando ao fracasso do tratamento endodôntico. Este acidente ocorreu durante o tratamento de canal e é influenciado pela técnica de instrumentação, tipo de sistema, agentes quelantes e solução irrigadora (6, 13).

Esse estudo avaliou o potencial de influência das soluções irrigadoras no transporte do canal radicular durante a instrumentação. A hipótese foi baseada em estudos prévios que mostraram mudanças na microdureza dentinária, pelo uso de soluções irrigadoras, especialmente após a aplicação de agentes quelantes (22-24). A hipótese nula testada foi rejeitada uma vez que o uso de agentes quelantes não aumentou o transporte do canal radicular.

Esforços foram feitos no pareamento das amostras afim de obter grupos com anatomia similar. Portanto raízes mesiais de molares inferiores semelhantes com dois canais e forames distintos foram divididas em grupos, considerando o diâmetro apical (compatível com uma lima K 15), volume da porção apical (1,26-8,11), comprimento do canal (19 a 21 mm) e o ângulo de curvatura do canal (25° a 40°).

O protocolo mais amplamente utilizado para a preparação do canal radicular tem sido a utilização de hipoclorito de sódio e EDTA. No entanto, os estudos mostraram efeitos prejudiciais a dentina (6, 23, 25) de modo a reduzir estes efeitos tem sido testado usando hipoclorito de sódio associado com HEBP (1, 3, 6, 7-12), um agente quelante fraco com menos danos a dentina (7, 8) e tem a vantagem de ser capaz de ser misturado com o NaOCl (3, 7), sem interferir com a sua capacidade de dissolução tecidual (3, 9) e propriedade antimicrobiana (9-11) redução a formação da camada de esfregaço e o tempo de trabalho clínico.

Foi demonstrado que a utilização de EDTA como agente irrigante durante o preparado com limas manuais de NiTi aumenta o transporte apical quando comparado ao canal irrigado com água destilada, provavelmente pela capacidade de remoção de íons cálcio da dentina pelo EDTA, alterando as propriedades físicas e mecânicas, tornando a dentina mais mole e mais facilmente removível (13). Mesmo quando 1-hidroxietilideno-1,1-bifosfonato (HELP), um agente quelante fraco com menos danos a dentina (7, 8), foi utilizado como irrigante, em conjunção com Protaper Universal o transporte apical foi observado (6).

Os grupos experimentais desta pesquisa, independentemente da solução de irrigação usada, mostraram o mesmo comportamento em relação ao desvio da trajetória original do canal. Os valores medianos de transporte variaram entre 0.05 a 0.06 no terço apical, de 0.12 a 0.18 e 0.21 a 0.23 nos níveis médio e cervical, respectivamente. A relevância clínica de valor máximo de transporte permanece questionável, valores até 0,10 mm foram considerados aceitáveis (26), porém foi relatado o impacto negativo do transporte apical de mais de 0,3 mm na obturação do canal radicular, com valores de transporte > 0.3 mostrando mais frequentemente infiltração (27). No terço apical, todas as amostras apresentaram valores abaixo deste limite. Dez amostras do terço cervical (3 G1, 3 G2, 2 G3 e 2 G4) mostraram transporte acima de 0,3 mm em relação ao terço médio transporte para além deste limite foram observadas em duas amostras (2 G4).

Ao comparar estudos que avaliam a influência de soluções de irrigação sobre o desvio do canal radicular, diferenças são encontradas no tipo de análise, o tipo de instrumento e o tempo ação das soluções (6, 13, 14). A instrumentação manual e os sistemas de rotação requerem mais tempo de instrumentação em comparação com sistemas de lima única reciprocantes. Assim, há um maior tempo de contato entre a solução irrigadora e a dentina, e a ação prolongada de agentes quelantes e do hipoclorito de sódio pode resultar em maior descalcificação da dentina (28) e no aumento da área para a ação de agentes quelantes devido à desproteinização do canal causada pelo NaOCl o que facilita o corte devido a diminuição da microdureza dentinária (6).

Nesse estudo o transporte do canal radicular ocorreu em todos os grupos sem influência do tipo de irrigante. Sendo resultado da ação da lima Primary Wave One. Os terços cervial ($p<0.05$) e médio ($p<0.05$) mostrou o transporte mais elevada em comparação ao terço apical. A direção de transporte nos terços cervical e médio ocorreu no sentido da furca e no terço apical para mesial. Os resultados corrobaram com um estudo prévio que avaliou através de microtomografia computadorizada o transporte do canal radicular instrumentado usando Primary Wave One (Dentsply Maillefer) com e sem alargamento cervial e apical prévio, mostrando que a direção do transporte no terço cervical e médio foi para a fura, enquanto no terço apical foi transportado para a mesial, sem diferença estatisticamente significante entre os níveis cervical e médio do canal (16).

A maior remoção de dentina na região de bifurcação no terço cervical também foi relatada usando o Protaper Universal, K3 (29), aço inoxidável (30), Mtwo e Reciproc (31). A anatomia do canal pode explicar esse efeito pela presença de projeção dentinária cervical que força o instrumento em direção inversa causando o aumento da tensão e desgaste na região de bifurcação no nível médio-coronal (29).

A diferença entre os terços também pode ser devido as características do sistema usado. Wave One system é feita de liga de níquel-titânio M-Wire, tem diferentes aumento de Taper progressivo, ponta inativa e seção-transversal variada ao longo da parte ativa (17, 32), convexa triangular na porção média e coronária do instrumento e convexa triangular modificada com guia radial na porção apical (16), o que dá ao instrumento capacidade mais centralizado (17, 32). O arquivo de maior diâmetro nas porções médio e cervical fornece menor flexibilidade em relação à porção apical pode ser responsável pelo maior desvio nos terços cervical e médio, quando comparado ao terço apical.

Assim este estudo mostrou que o uso do hipoclorito de sódio e os agentes irrigantes usados em diferentes protocolos de irrigação durante a instrumentação não aumentaram significativamente o transporte do canal radicular. Sendo o transporte associado a ação da lima Primary Wave One, mostrando-se maior no terço cervical e médio quando comparado ao terço apical e sendo direcionado para a zona de bifurcação nos terços cervical e médio e para a mesial no terço apical.

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Legenda de Figura

Figura 1. Secção de micro-CT da região apical de canais não instrumentatos (A) e instrumentados (B) para avaliação do transporte do canal radicular.

TABELA 1. Valores de mediana, mínimo (Min) e máximo (Max) para comparação do transporte do canal radicular em milímetros.

| Grupos | Cervical | Médio | Apical |
|---|---|---|---|
| | Mediana (Min – Max) | Mediana (Min – Max) | Mediana (Min – Max) |
| G1 | 0.21 (0.13 - 0.43) | 0.16 (0.09 - 0.24) | 0.05 (0.01 - 0.12) |
| G2 | 0.22 (0.09 - 0.45) | 0.12 (0.04 - 0.22) | 0.05 (0.03 - 0.08) |
| G3 | 0.23 (0.16 – 0.35) | 0.18 (0.07- 0.27) | 0.05 (0.01 - 0.10) |
| G4 | 0.23 (0.05 – 0.33) | 0.15 (0.09- 0.30) | 0.06 (0.01 - 0.12) |
| p-valor | 0.8152 | 0.2308 | 0.5899 |

G1 – solução salina; G2 – NaOCl; G3 – NaOCl associado com HEBP; G4 –NaOCl alternado com EDTA.

TABELA 2. Rank diferença e p-valor (Kruskal-Wallis) para comparar o transporte do canal radicular entre os níveis do canal radicular.

| Nível | G1 | | G2 | | G3 | | G4 | |
|---|---|---|---|---|---|---|---|---|
| | Rank diference | p-valor | Rank diference | p-valor | Rank diference | p-valor | Rank diference | p-valor |
| Cervical x Médio | 9.18 | Ns | 11.60 | ns | 9.43 | ns | 6.36 | ns |
| Cervical x Apical | 24.25 | <0.05 | 25.04 | <0.05 | 25.29 | <0.05 | 22.04 | <0.05 |
| Médio x Apical | 15.07 | <0.05 | 13.43 | <0.05 | 15.86 | <0.05 | 15.68 | <0.05 |

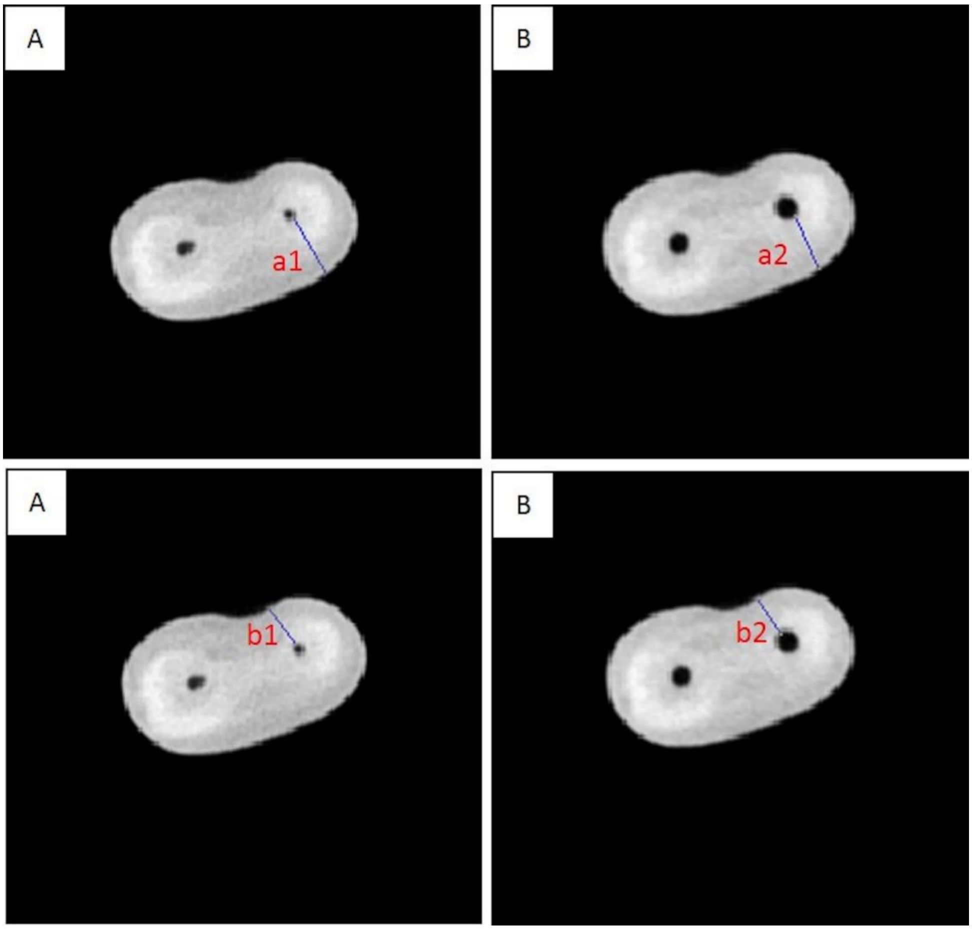
G1 – solução salina; G2 – NaOCl; G3 – NaOCl associado com HEBP; G4 –NaOCl alternado com EDTA.

TABELA 3. Direção do transporte do canal radicular em cada nível do canal radicular.

| Groups | Direction | Cervical | | Middle | | Apical | |
|---|---|---|---|---|---|---|---|
| | | n | % | n | % | n | % |
| G1 | Mesial | 19 | 45,20 | 13 | 30,95 | 25 | 59,52 |
| | Distal | 22 | 52,38 | 29 | 69,05 | 14 | 33,33 |
| | Nulo | 1 | 2,38 | 0 | 0,00 | 3 | 7,14 |
| G2 | Mesial | 15 | 35,71 | 11 | 26,19 | 25 | 59,52 |
| | Distal | 27 | 64,29 | 30 | 71,43 | 16 | 38,10 |
| | Nulo | 0 | 0,00 | 1 | 2,38 | 1 | 2,38 |
| G3 | Mesial | 18 | 42,86 | 10 | 23,81 | 23 | 54,76 |
| | Distal | 23 | 54,76 | 32 | 76,19 | 19 | 45,24 |
| | Nulo | 1 | 2,38 | 0 | 0,00 | 0 | 0,00 |
| G4 | Mesial | 20 | 47,62 | 17 | 40,48 | 22 | 52,38 |
| | Distal | 21 | 50,00 | 25 | 59,52 | 18 | 42,86 |
| | Nulo | 1 | 2,38 | 0 | 0,00 | 2 | 4,76 |

G1 – solução salina; G2 – NaOCl; G3 – NaOCl associado com HEBP; G4 –NaOCl alternado com EDTA.

Figura 1. Secção de micro-CT da região apical de canais não instrumentatos (A) e instrumentados (B) para avaliação do transporte do canal radicular.



# APÊNDICE A- NORMAS PARA PUBLICAÇÃO DO JOE

Guidelines for Publishing Papers in the JOE

Writing an effective article is a challenging assignment. The following guidelines are provided to assist authors in submitting manuscripts.

The *JOE* publishes original and review articles related to the scientific and applied aspects of endodontics. Moreover, the *JOE* has a diverse readership that includes full-time clinicians, full-time academicians, residents, students and scientists. Effective communication with this diverse readership requires careful attention to writing style.

**General Points on Composition**
**Organization of Original Research Manuscripts**
**Manuscripts Category Classifications and Requirements**
**Available Resources**

1. **General Points on Composition**
   1. Authors are strongly encouraged to analyze their final draft with both software (*e.g.*, spelling and grammar programs) and colleagues who have expertise in English grammar. References listed at the end of this section provide a more extensive review of rules of English grammar and guidelines for writing a scientific article. Always remember that clarity is the most important feature of scientific writing. Scientific articles must be clear and precise in their content and concise in their delivery since their purpose is to inform the reader. The Editor reserves the right to edit all manuscripts or to reject those manuscripts that lack clarity or precision, or have unacceptable grammar or syntax. The following list represents common errors in manuscripts submitted to the*JOE*:
   2. The paragraph is the ideal unit of organization. Paragraphs typically start with an introductory sentence that is followed by sentences that describe additional detail or examples. The last sentence of the paragraph provides conclusions and forms a transition to the next paragraph. Common problems include one-sentence paragraphs, sentences that do not develop the theme of the paragraph (see also section "c" below), or sentences with little to no transition within a paragraph.
   3. Keep to the point. The subject of the sentence should support the subject of the paragraph. For example, the introduction of authors' names in a sentence changes the subject and lengthens the text. In a paragraph on sodium hypochlorite, the sentence, "In 1983, Langeland et al., reported that sodium hypochlorite acts as a lubricating factor during instrumentation and helps to flush debris from the root canals" can be edited to: "Sodium hypochlorite acts as a lubricant during instrumentation and as a vehicle for flushing the generated debris (Langeland et al., 1983)." In this example, the paragraph's subject is sodium hypochlorite and sentences should focus on this subject.
   4. Sentences are stronger when written in the active voice, *i.e.*, the subject performs the action. Passive sentences are identified by the use of passive verbs such as "was," "were," "could," etc. For example: "Dexamethasone was found in this study to be a factor that was associated with reduced inflammation," can be edited to: "Our results demonstrated that dexamethasone reduced inflammation." Sentences written in a direct and active voice are generally more powerful and shorter than sentences written in the passive voice.

5. Reduce verbiage. Short sentences are easier to understand. The inclusion of unnecessary words is often associated with the use of a passive voice, a lack of focus or run-on sentences. This is not to imply that all sentences need be short or even the same length. Indeed, variation in sentence structure and length often helps to maintain reader interest. However, make all words count. A more formal way of stating this point is that the use of subordinate clauses adds variety and information when constructing a paragraph. (This section was written deliberately with sentences of varying length to illustrate this point.)
6. Use parallel construction to express related ideas. For example, the sentence, "Formerly, endodontics was taught by hand instrumentation, while now rotary instrumentation is the common method," can be edited to "Formerly, endodontics was taught using hand instrumentation; now it is commonly taught using rotary instrumentation." The use of parallel construction in sentences simply means that similar ideas are expressed in similar ways, and this helps the reader recognize that the ideas are related.
7. Keep modifying phrases close to the word that they modify. This is a common problem in complex sentences that may confuse the reader. For example, the statement, "Accordingly, when conclusions are drawn from the results of this study, caution must be used," can be edited to "Caution must be used when conclusions are drawn from the results of this study."
8. To summarize these points, effective sentences are clear and precise, and often are short, simple and focused on one key point that supports the paragraph's theme.
9. Authors should be aware that the *JOE* uses iThenticate, plagiarism detection software, to assure originality and integrity of material published in the *Journal*. The use of copied sentences, even when present within quotation marks, is highly discouraged. Instead, the information of the original research should be expressed by new manuscript author's own words, and a proper citation given at the end of the sentence. Plagiarism will not be tolerated and manuscripts will be rejected, or papers withdrawn after publication based on unethical actions by the authors. In addition, authors may be sanctioned for future publication.

2. **Organization of Original Research Manuscripts**

***Please Note:*** *All abstracts should be organized into sections that start with a one-word title (in bold),* i.e., *Introduction, Methods, Results, Conclusions, etc., and should not exceed more than 250 words in length.*

1. **Title Page:** The title should describe the major emphasis of the paper. It should be as short as possible without loss of clarity. Remember that the title is your advertising billboard—it represents your major opportunity to solicit readers to spend the time to read your paper. It is best not to use abbreviations in the title since this may lead to imprecise coding by electronic citation programs such as PubMed (*e.g.*, use "sodium hypochlorite" rather than NaOCl). The author list must conform to published standards on authorship (see authorship criteria in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals at***www.icmje.org***). The manuscript title, name and address (including email) of one author designated as the corresponding author. This author will be responsible for editing proofs and ordering reprints when applicable. The contribution of each author should also be highlighted in the cover letter.
2. **Abstract:** The abstract should concisely describe the purpose of the study, the hypothesis, methods, major findings and conclusions. The abstract should describe the new contributions made by this study. The word limitations (250 words) and

the wide distribution of the abstract (*e.g.*, PubMed) make this section challenging to write clearly. This section often is written last by many authors since they can draw on the rest of the manuscript. Write the abstract in past tense since the study has been completed. Three to ten keywords should be listed below the abstract.

3. **Introduction:** The introduction should briefly review the pertinent literature in order to identify the gap in knowledge that the study is intended to address and the limitations of previous studies in the area. The purpose of the study, the tested hypothesis and its scope should be clearly described. Authors should realize that this section of the paper is their primary opportunity to establish communication with the diverse readership of the *JOE*. Readers who are not expert in the topic of the manuscript are likely to skip the paper if the introduction fails to succinctly summarize the gap in knowledge that the study addresses. It is important to note that many successful manuscripts require no more than a few paragraphs to accomplish these goals. Therefore, authors should refrain from performing extensive review or the literature, and discussing the results of the study in this section.
4. **Materials and Methods:** The objective of the materials and methods section is to permit other investigators to repeat your experiments. The four components to this section are the detailed description of the materials used and their components, the experimental design, the procedures employed, and the statistical tests used to analyze the results. The vast majority of manuscripts should cite prior studies using similar methods and succinctly describe the essential aspects used in the present study. Thus, the reader should still be able to understand the method used in the experimental approach and concentration of the main reagents (*e.g.*, antibodies, drugs, etc.) even when citing a previously published method. The inclusion of a "methods figure" will be rejected unless the procedure is novel and requires an illustration for comprehension. If the method is novel, then the authors should carefully describe the method and include validation experiments. If the study utilized a **commercial product**, the manuscript must state that they either followed manufacturer's protocol *or*specify any changes made to the protocol. If the study used an ***in vitro* model** to simulate a clinical outcome, the authors must describe experiments made to validate the model, or previous literature that proved the clinical relevance of the model. Studies on **humans** must conform to the Helsinki Declaration of 1975 and state that the institutional IRB/equivalent committee(s) approved the protocol and that informed consent was obtained after the risks and benefits of participation were described to the subjects or patients recruited. Studies involving **animals** must state that the institutional animal care and use committee approved the protocol. The statistical analysis section should describe which tests were used to analyze which dependent measures; p-values should be specified. Additional details may include randomization scheme, stratification (if any), power analysis as a basis for sample size computation, drop-outs from clinical trials, the effects of important confounding variables, and bivariate versus multivariate analysis.
5. **Results:** Only experimental results are appropriate in this section (*i.e.*, neither methods, discussion, nor conclusions should be in this section). Include only those data that are critical for the study, as defined by the aim(s). Do not include all available data without justification; any repetitive findings will be rejected from publication. All Figures, Charts and Tables should be described in their order of numbering with a brief description of the major findings. Author may consider the use of supplemental figures, tables or video clips that will be published online.

Supplemental material is often used to provide additional information or control experiments that support the results section (*e.g.*, microarray data).

6. **Figures:** There are two general types of figures. The first type of figures includes photographs, radiographs or micrographs. Include only essential figures, and even if essential, the use of composite figures containing several panels of photographs is encouraged. For example, most photo-, radio- or micrographs take up one column-width, or about 185 mm wide X 185 mm tall. If instead, you construct a two columns-width figure (*i.e.*, about 175 mm wide X 125 mm high when published in the *JOE*), you would be able to place about 12 panels of photomicrographs (or radiographs, etc.) as an array of four columns across and three rows down (with each panel about 40 X 40 mm). This will require some editing to emphasize the most important feature of each photomicrograph, but it greatly increases the total number of illustrations that you can present in your paper. Remember that each panel must be clearly identified with a letter (*e.g.*, "A," "B," etc.), in order for the reader to understand each individual panel. Several nice examples of composite figures are seen in recent articles by Jeger et al (J Endod 2012;38:884–888); Olivieri et al., (J Endod 2012;38:1007 1011); Tsai et al (J Endod 2012;38:965–970). Please note that color figures may be published at no cost to the authors and authors are encouraged to use color to enhance the value of the illustration. Please note that a multipanel, composite figure only counts as one figure when considering the total number of figures in a manuscript (see section 3, below, for maximum number of allowable figures).

   The second type of figures are graphs (*i.e.*, line drawings including bar graphs) that plot a dependent measure (on the Y axis) as a function of an independent measure (usually plotted on the X axis). Examples include a graph depicting pain scores over time, etc. Graphs should be used when the overall trend of the results are more important than the exact numerical values of the results. For example, a graph is a convenient way of reporting that an ibuprofen-treated group reported less pain than a placebo group over the first 24 hours, but was the same as the placebo group for the next 96 hours. In this case, the trend of the results is the primary finding; the actual pain scores are not as critical as the relative differences between the NSAID and placebo groups.
7. **Tables:** Tables are appropriate when it is critical to present exact numerical values. However, not all results need be placed in either a table or figure. For example, the following table may not be necessary:

   Instead, the results could simply state that there was no inhibition of growth from 0.001-0.03% NaOCl, and a 100% inhibition of growth from 0.03-3% NaOCl (N=5/group). Similarly, if the results are not significant, then it is probably not necessary to include the results in either a table or as a figure. These and many other suggestions on figure and table construction are described in additional detail in Day (1998).
8. **Discussion:** This section should be used to interpret and explain the results. Both the strengths and weaknesses of the observations should be discussed. How do these findings compare to the published literature? What are the clinical implications? Although this last section might be tentative given the nature of a particular study, the authors should realize that even preliminary clinical implications might have value for the clinical readership. Ideally, a review of the

potential clinical significance is the last section of the discussion. What are the major conclusions of the study? How does the data support these conclusions

9. **Acknowledgments:** All authors must affirm that they have no financial affiliation (*e.g.*, employment, direct payment, stock holdings, retainers, consultantships, patent licensing arrangements or honoraria), or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript, nor have any such arrangements existed in the past three years. Any other potential conflict of interest should be disclosed. Any author for whom this statement is not true must append a paragraph to the manuscript that fully discloses any financial or other interest that poses a conflict. Likewise the sources and correct attributions of all other grants, contracts or donations that funded the study must be disclosed
10. **References:** The reference style follows Index Medicus and can be easily learned from reading past issues of the *JOE*. The *JOE* uses the Vancouver reference style, which can be found in most citation management software products. Citations are placed in parentheses at the end of a sentence or at the end of a clause that requires a literature citation. Do not use superscript for references. Original reports are limited to 35 references. There are no limits in the number of references for review articles.

3. **Manuscripts Category Classifications and Requirements**

Manuscripts submitted to the *JOE* must fall into one of the following categories. The abstracts for all these categories would have a maximum word count of 250 words:

1. CONSORT Randomized Clinical Trial-Manuscripts in this category must strictly adhere to the Consolidated Standards of Reporting Trials-CONSORT- minimum guidelines for the publication of randomized clinical trials. These guidelines can be found at ***www.consort-statement.org/***. These manuscripts have a limit of 3,500 words, [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.
2. Review Article-Manuscripts in this category are either narrative articles, or systematic reviews/meta-analyses. Case report/Clinical Technique articles even when followed by extensive review of the literature will should be categorized as “Case Report/Clinical Technique”. These manuscripts have a limit of 3,500 words, [including abstract, introduction, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.
3. Clinical Research (*e.g.*, prospective or retrospective studies on patients or patient records, or research on biopsies, excluding the use of human teeth for technique studies). These manuscripts have a limit of 3,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.
4. Basic Research Biology (animal or culture studies on biological research on physiology, development, stem cell differentiation, inflammation or pathology). Manuscripts that have a primary focus on biology should be submitted in this category while manuscripts that have a primary focus on materials should be submitted in the Basic Research Technology category. For example, a study on cytotoxicity of a material should be submitted in the Basic Research Technology category, even if it was performed in animals with histological analyses. These manuscripts have a limit of 2,500 words [including abstract, introduction, materials

and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures or 4 tables*.

5. Basic Research Technology (Manuscripts submitted in this category focus primarily on research related to techniques and materials used, or with potential clinical use, in endodontics). These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 3 figures and tables *.
6. Case Report/Clinical Technique (*e.g.*, report of an unusual clinical case or the use of cutting-edge technology in a clinical case). These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures or tables*.

* Figures, if submitted as multipanel figures must not exceed 1 page length. Manuscripts submitted with more than the allowed number of figures or tables will require approval of the *JOE* Editor or associate editors. If you are not sure whether your manuscript falls within one of the categories above, or would like to request preapproval for submission of additional figures please contact the Editor by email at ***jendodontics@uthscsa.edu***.

Importantly, adhering to the general writing methods described in these guidelines (and in the resources listed below) will help to reduce the size of the manuscript while maintaining its focus and significance. Authors are encouraged to focus on only the essential aspects of the study and to avoid inclusion of extraneous text and figures. The Editor may reject manuscripts that exceed these limitations.

**Available Resources:**

Strunk W, White EB. The Elements of Style. Allyn & Bacon, 4th ed, 2000, ISBN 020530902X.

Day R. How to Write and Publish a Scientific Paper. Oryx Press, 5th ed. 1998. ISBN 1-57356-164-9.

Woods G. English Grammar for Dummies. Hungry Minds:NY, 2001 (an entertaining review of grammar).

Alley M. The Craft of Scientific Writing. Springer, 3rd edition 1996 SBN 0-387-94766-3.

Alley M. The Craft of Editing. Springer, 2000 SBN 0-387-98964-1.